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Anatomical Study for Vegetative Organs of *Pelargonium graveolens* L.'Hér Cultivated in Iraq

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Abstract

The current investigation concluded an anatomical study of the cultivated species *Pelargonium graveolens* L. 'Hér belong to the Geraniaceae family and which cultivated as an ornamental plant, fresh specimen were collected from different region in Baghdad ,the study vegetative parts (leaf, stem and petiole) in details ,the upper and lower epidermis of leaf and epidermis of stem also studied, ordinary epidermal cells and stomatal complexes studied in term of shapes and dimensions ,the result showed that stomatal in leaf and stem is anomocytic type, the cross sections of the vegetative parts prepared by microtome and free hand sectioning. The tissues of these parts include the number and the type of layers and thickness of each one , the result showed the presence of druses crystals in parenchyma cells of the petiole, stem and leaf. Vistur type of this organs also studied and it comprises of glandular and non-glandular trichomes which occur in different types and lengths.

Keywords: *Pelargonium*, anatomical study, leaf, petiole, stem, indumentum.

1. Introduction

Pelargonium graveolens L. 'Hér belongs to the Geraniaceae family and is a species known as rose-scented geranium. Genus Pelargonium has more than 200 species. It originated in South Africa, where over 700 varieties of cultivated Geranium exist [1]. The plant is erect, multi-branched shrub that grows up to 1.5 m and has a spread of 1 m. [2]. The young stems are villous to density villous and herbaceous, but they become glabrous and woody with age. The leaves are palmatipartite to pinnatisect with irregularly pinnatipartite segments and are soft to touch. The margins of the segments are more or less revolute. White to pinkish-purple flowers is borne in 3-7 flowered pseudo-umbels [3].

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The essential oil of *Pelargonium graveolens* has antifungal and antioxidant activity [4]. It also has antibacterial and insecticidal properties. The study of Jaradat *et al.* [5] demonstrates that this plant represents a rich source of bioactive substances that can be further investigated and authenticated for their medicinal potential. Boukhris *et al*. [6] demonstrated that administration of the essential oil of *P. graveolens* may be helpful in the prevention of diabetic complications associated with oxidative stress. Obeid and Jaber [7] studied the chemical composition and antioxidant activity of *P. graveolens.*

Pelargonium species and related genera in Geraniaceae have been studied anatomically in many investigations, such as the study of Van der Walt and Demarne [3] for Pelargonium graveolens and P. radens in terms of their morphology and essential oils. The study included the trichomes in leaves, while Boukhris et al. [8] investigated the biological and anatomical characteristics of Pelargonium graveolens grown in the south of Tunisia, Salama et al. [9] studied the petiole and lamina of six species, including Pelargonium graveolens, which grows in Egypt; histo-anatomical studies on the vegetative and reproductive organs of Pelargonium roseum growing in Romania were conducted by Gâlea et al. [10]; Lancu et al. [11] also studied some Pelargonium species cultivated in Romania.

The aim of this study is to describe anatomical features of vegetative organs for Pelargonium graveolens, which is cultivated in Baghdad, Iraq.

2. Materials and Methods

Fresh specimens were collected from different regions in Baghdad, the upper and lower surface epidermis of blade leaf and stem were prepared by using stripping off using forcipes then transferred to a slide containing a drop of safranin -glycerine, then covered with a cover slide and examined under light microscope to study stomatal complex and ordinary epidermal cells [12]. *The* replica method was also used for taking imprints [13]. The stomatal index (%) was calculated according to the following formula: (S/S+E) ×100 where S and E are the numbers of stomata and epidermis, respectively, in the microscopic view field [14]. The cross sections of the stem, petiole, and leaf were prepared by microtome and hand sections and stained by toluidine blue, then examined under the microscope. The indumentum was also studied, and dimensions were determined by using an ocular micrometer.

3. Results

Microscopic analysis of the surface epidermis of the leaf showed that the ordinary epidermal cells on the adaxial and abaxial surfaces had a polygonal shape, while their walls were undulate; the average dimension was (88.4 ×26) mm and (50.2 ×33.8) mm, respectively as shown in **Table 1** and **Figure 1**.

The leaf is amphistomatal and anomocytic, and the average dimensions of stomatal complexes were (37.7×26.3) mm and (30.3×29.2) mm in the upper and lower surfaces, respectively. The stomatal index varied in the upper and lower epidermis, reaching 8 in the upper epidermis and 29.2 in the lower epidermis (**Table 1**, **Figuer1**).

The vertical section of the leaf showed that the blade leaf was bifacial, with a palisade layer on the upper side and a spongy layer on the lower side. The section of lamina consists of the upper epidermis (30) mm, which is covered by the cuticle (30) mm. The epidermis is followed by the mesophyll, which consists of the palisade layer and the spongy layer. The palisade layer is composed of one row of oblong cells, while the spongy layer is composed of 2-3 rows of cells

covered by the cuticle. Between two layers of mesophyll, there are parenchymatous cells containing drusen crystals, which are composed of calcium oxalate (**Table 2, Figure 1**).

As shown in **Table 3** and **Figure 1**, the midrib region has a semicircular outline. Collenchyma tissue and parenchyma tissue are located under the upper and lower epidermis. There are two central vascular bundles. The upper one is semicircular and smaller than the other vascular bundles, which are crescent-shaped (190.25123.06 mm). The thickness of xylem and phloem is 71.5 mm and 29.9 mm, respectively. The number of xylem columns is 24-28, and the number of xylem elements is 1-4.

The petiole has a semicircular outline shape with a depression at the top of the section (Figure 2). It consists of the epidermis, covered by a toothed cuticle; the cortex located under the epidermis, which consists of one row of collenchyma, followed by 2-3 rows of chlorenchyma layers; the ordinary parenchyma layer follows the chlorenchyma layer, which also consists of 2-3 rows; druses crystal are found in this layer; beneath the parenchyma layer, there is a continuous ring of fiber, A vascular cylinder consists of 7-9 vascular bundles in a semi-triangular shape in three different sizes. The largest one in the top of the petiole, three medium-size bundles alternate with 3-5 small ones, and pith occupied the center of the petiole, which is composed of parenchyma cells (**Table 4**, **Figure 2**)

The surface epidermis of the stem is composed of oblong-polygonal ordinary epidermal cells with straight, oblique walls. Stomata occur in the stem and its anomocytic type as well as in leaves, and the stomatal index reaches 3.77. (Table 5; Figure 3)

Stem transverse section circular outline, anatomical structure form of epidermis composed of one row cells covered by toothed cuticle, cortex located below epidermis which consist of one row of collenchyma, 1-2 rows of chlorenchyma, 6-7 rows of parenchyma druses crystal found in this layer, fibers forms continuous ring above vascular cylinder which consist of 9-12 bundles in three different size , Keshavarzi et al.(2016) mentioned that the vascular bundles in stem occur in different size, vascular bundle compose of phloem and xylem, cambium obvious between phloem and xylem, this agreement with Gâlea et al. (2017), pith occupied the center of stem consist of parenchyma cell. (table 6; figure 4)

Indumentum concluded that both glandular and non glandular hairs of different types, as follows, were observed in different vegetative parts:

Unicellular non glandular hairs occur in all vegetative parts of the plant at different average lengths (414.75) mm in the leaf, (255.5) mm in the petiole, and (346.5) mm in the stem) (Table 7, Figure 5-A).

Multicellular non glandular hairs formed by two cells are observed in the leaf at two different lengths (378) mm and (647) mm. (Table 7, Figure 5-B)

Multicellular, non glandular hairs formed by 3–4 cells occur in the stem, their length reaching (895.62) mm. (Table 7, Figure 5-C)

Glandular trichomes have at the top a secretory cell covered with a cuticle. Below the cuticle, in the secretion of the cell, essential oils are decomposed into different types:

Sessile glandular hairs occur in the leaf; the head diameter was (11.8) mm (Table 7, Figure 5-D).

Stalked glandular hairs, the stalk composed of one cell, occur in the leaf; stalk length is 9.5 mm; and the head is semispherical. (Table 7, Figure 5-E), this hair was also observed in the stem; the stalk length was (12.13) mm. On the stem, there is the same type, but the hair has a narrow neck between the average lengths of the stalk (16.46) mm. (Table, Figure 5-F)

Stalked glandular hairs, stalk formed by 2-3 cells, the head having a spherical or irregular shape , stalk length reaching 47 mm (**Table 7, Figure 5-G**).

4. Discussion

The ordinary epidermal cells on the adaxial and abaxial sides of the leaf had a polygonal shape. Salimpour *et al.* [15] reported in their study of ten species of *Geranium* in Iran that some species had the polygonal shape of epidermal cells.

The result shows that the leaf is amphistomatal and anomocytic. This agrees with [16], [9], [10], [11], and [15]. The study showed the occurrence of druses crystals in parenchyma cells between the palisade layer and spongy, which are composed of calcium oxalate. This crystal was reported in the studies carried out by [9], [10], and [17].

The petiole cortex contains a chlorenchyma layer, and this agrees with the study of Marias [18]. She also noted that the presence of a chlorenchyma petiole is evidence of its participation in the photosynthesis process. The ordinary parenchyma layer follows the chlorenchyma layer, and druse crystals are found in this layer. This is also in agreement with Marias [18] and Galea *et al.* [10]: beneath the parenchyma layer,

There is a continuous ring of fiber in the vascular cylinder, composed of 7-9 vascular bundles in semi-triangular shape in three different sizes, the largest one in the top of the petiole, and this agrees with Lancu *et al.* [11], three medium-sized bundles alternate with 3-5 small ones. Keshavarzi *et al.* [19] also refer to the variation in bundle size in the petiole.

In the stem transverse section, fibers form a continuous ring above the vascular cylinder, which consists of 9–12 bundles in three different sizes. Keshavarzi *et al*. [19] mentioned that the vascular bundles in the stem occur in different sizes.

Indumentum concluded both glandular and non glandular hairs in different types. Many researchers reported glandular and non glandular hairs in their investigations, such as [8, 15, 17, 19, 20, 21, 22, and 23]. The current study agrees with Boukhris *et al*. [8] in that the highest length of non glandular hairs was recorded on the stem.

As superficial structures, non-glandular trichomes protect plant organs against multiple biotic and abiotic stresses. The protective and defensive roles of these epidermal appendages are crucial to developing organs and can be attributed to the excellent combination of suitable structural traits and chemical reinforcement in the form of phenolic compounds, primarily flavonoids. Due to the diffuse deposition of phenolic in the cell walls, trichomes provide protection against UV-B radiation by behaving as optical filters, screening out wavelengths that could damage sensitive tissues [24] while glandular hair structures present external cells empty of essential oils. When the cuticle is destroyed, the essential oils are dispersed into the atmosphere, which is a phenomenon that plays an important role in the attraction of insects [8]. Thus, glandular trichomes constitute a chemical barrier that reduces leaf ozone uptake and toxicity [25] and [26].

Table 1. Characters of surface epidermis of the leaf measured by micrometers (μm).

Upper epidermis					**Lower epidermis**				
Ordinary epidermal cells		**Stomatal complex**			**Ordinary epidermal cells**		**Stomatal complex**		
Length	Width	Length	Width	Stomatal index	Length	Width	Length	Width	Stomatal index
78-109 (88.9)	39-80.6 (56.7)	35.1-39 (37.7)	26-28.6 (26.8)	8	46.8-57.2 (50.26)	28.6-41.6 (33.8)	28.6-31.2 (30.33)	23.6-29 (26)	27.58

Number brackets represent average

Table 2. Characters of vertical section in the leaf (Lamina) measured by micrometers (μm).

Cuticle thickness		**Epidermis thickness**		**Palisade layer**		**Spongy layer**	
Upper surface	Lower surface	Upper surface	Lower surface	Thickness	Rows	Thickness	Rows
4.9-5.2 (5.02)	7.2-7.8 (7.45)	26-36.4 (32.0)	16.9-26 (22.1)	54.6-59.8 (58)	1	65-68.9 (66.3)	2-3

Number brackets represent average

Table 3. Characters of midrib region in the leaf measured by micrometers (μm).

Collenchyma thickness		**Parenchyma thickness**		**Central bundle**			
Upper	Lower	Upper	Lower	Dimension	Phloem thickness	Xylem thickness	Fiber thickness
25.7-28.6 (27.6)	36.9-78 (51.1)	59.4-62.4 (59.8)	208-239 (225.3)	468-494 (485.3)	28.6-31.2 (30.33)	59.8-65 (63.2)	15.6-18.2 (17.33)

Number brackets represent average

Table 4. Characters of cross section in petiole, measured by micrometers (µm).

Cuticle thickness	Epidermis thickness	Collenchyma thickness	Chlorenchyma thickness	Parenchyma thickness	No. of Bundles	Dimension	Fiber thickness	Phloem thickness	Vascular thickness	Xylem thickness	Pith diameter
5.2-6.5 (5.8)	18.2-20.8 (19.3)	21.3-23.4 (22.6)	95-104 (82.33)	91-130 (108.3)	7-9	257.4-312 ×195-286 (276×229.6)	28.6-39 (32.9)	26.46.8 (39)	43.95-52 (48.3)	54.2-78 (69.33)	735-787.5 (766.5)

Number brackets represent average

Table 5. Characters of cross section in stem , measured by micrometers (μm).

Stomata		Ordinary cell	Cuticle	Epidermis thickness	Collenchyma		Chlorenchyma		Parenchyma	
Dimension	Stomatal index	Dimension	thickness		Thickness	Rows	Thickness	Thickness	Thickness	Rows
41.6-44.2 ×410.8-520 (42.4)×(26.8)	3.77	88.4-135.2 ×13-18.2 (107.1)×(15)	4.6-5.2 (4.9)	21.9-23.4 (22.27)	27.8-30 (288)	1	44.2-70.2 (66.5)	203	182-208 -193.26	6-8

Table 6. Characters of cross section in stem, measured by micrometers (μm).

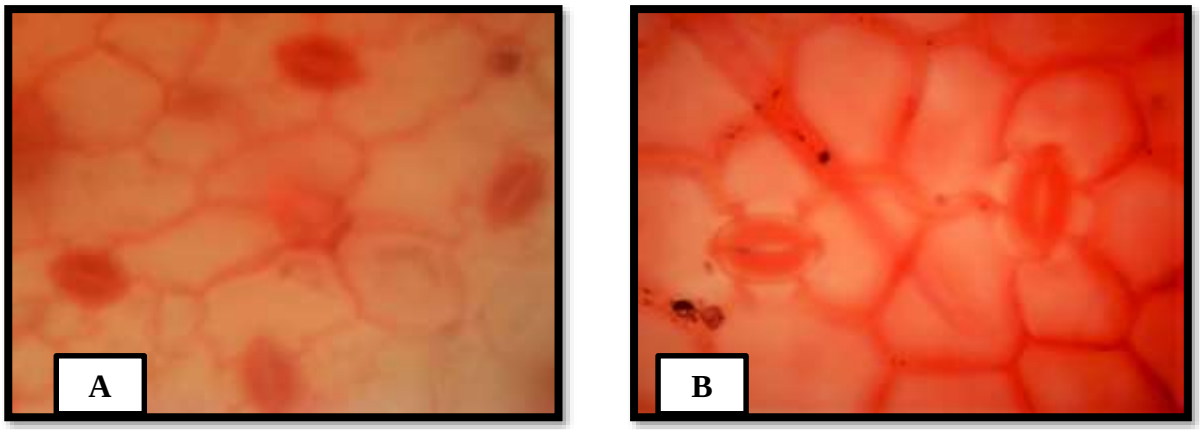
Bundle		Fiber thickness	Phloem thickness	Vascular cambium thickness	Xylem thickness	Pith diameter
Dimensions	**No.**					
364-416 ×410.8-520 (394.3)×(449.9)	9-12	52-104 (69.33)	62.4-69.74 (67.6)	52-57.2 (53.8)	208-239 (217.5)	1260-1365 (1323)

Number brackets represent average

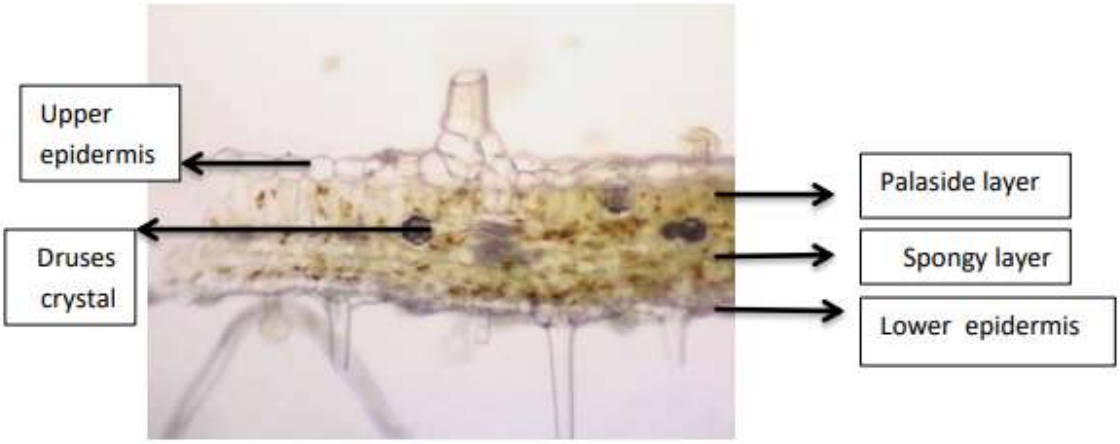
Table 7. Trichome characters measured by micrometer

Organ **Hair types**	**Petiole**	**Leaf**	**Stem**
A-Unicellular eglandular length	199.5-367.5 (255.5)	336-441(414.7)	309-367.5 (346.5)
B-Multicellular eglandular 2 cells, length,	-	(315-399) 378 (577.5-688.5) 647	-
A- Multicellular 3-4 cells,length	-	71.9-75.6 (74.2)	724.5-1.029 (895.6)
D-Sessile glandular hairs, head diameters	-	9.1-11.7 (11.18)	-
E- Stalked glandular hairs stalk cell , stalk length	14-65 (47.45)	(7.8-10.4) (9.5)	10.9-17.5 (12.13)
F- Stalked gland Stalk with neck	-	-	13-18.2 (16.4)
G-Stalked glandular hairs, stalk 2-3 cells	41.6- 65 (47.45)	-	-

The number between brackets represent average



Upper surface **Lower surface**



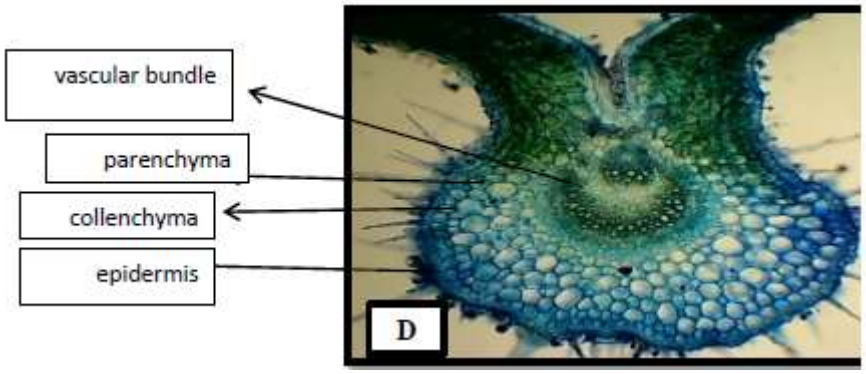


Figure 1. Characters of leaf: A and B surface epidermis 400x, C: Lamina, D: vein region. 100x

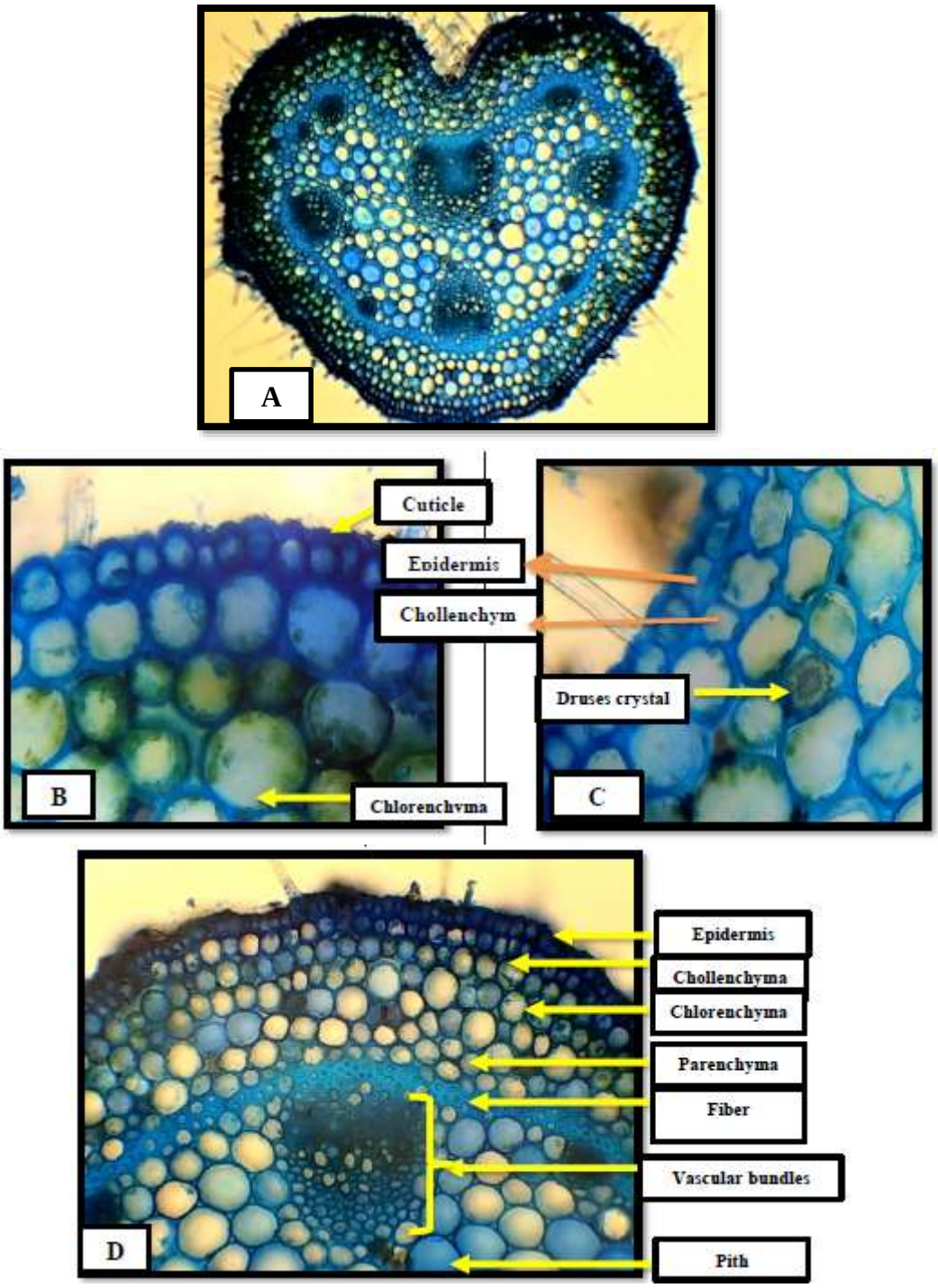


Figure 2. Cross section of Petiole .A -40X, B and C-400X, ,D-100X

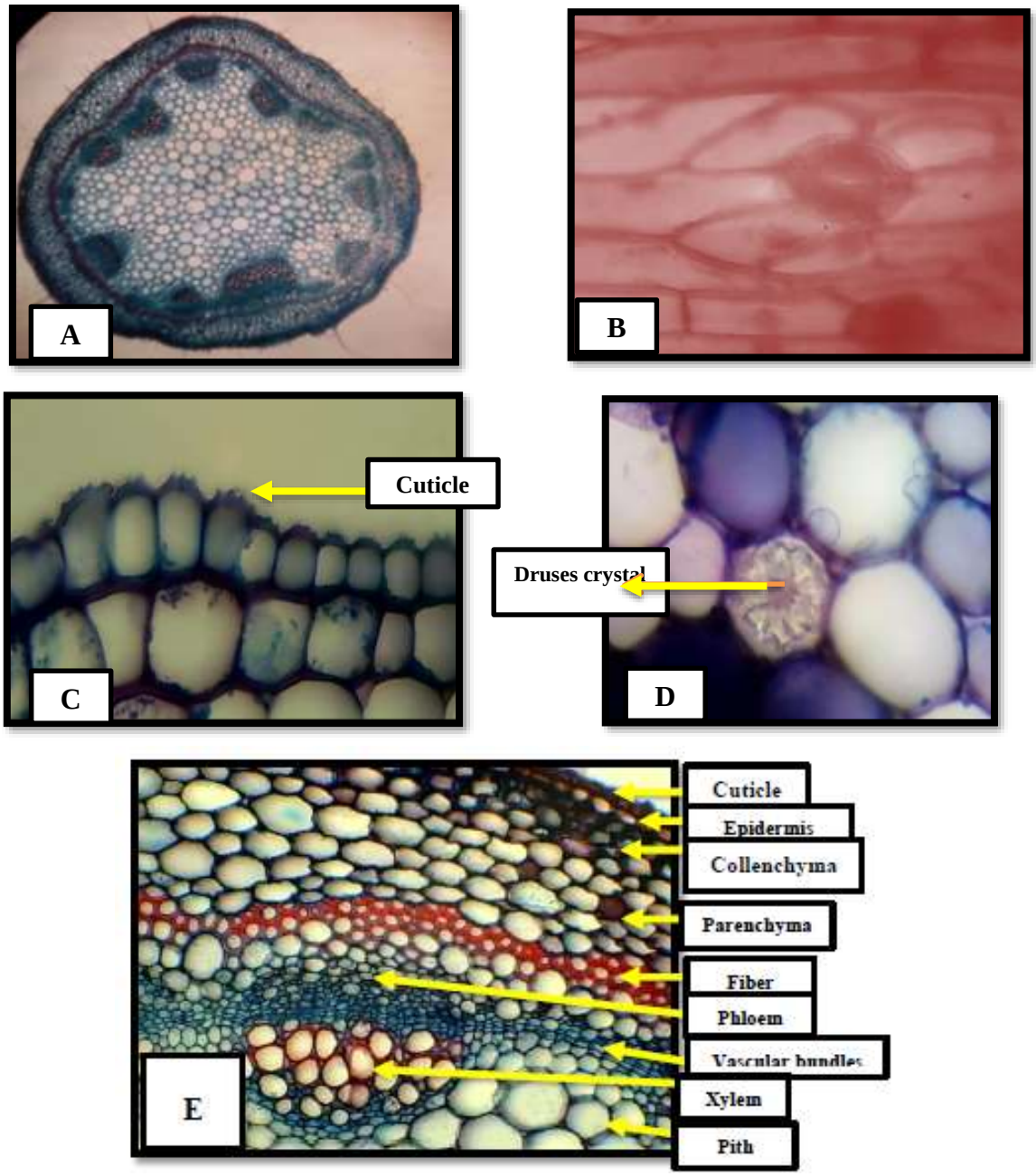


Figure 3. Cross section of stem.A-40X ,B-D-400X, E-100X

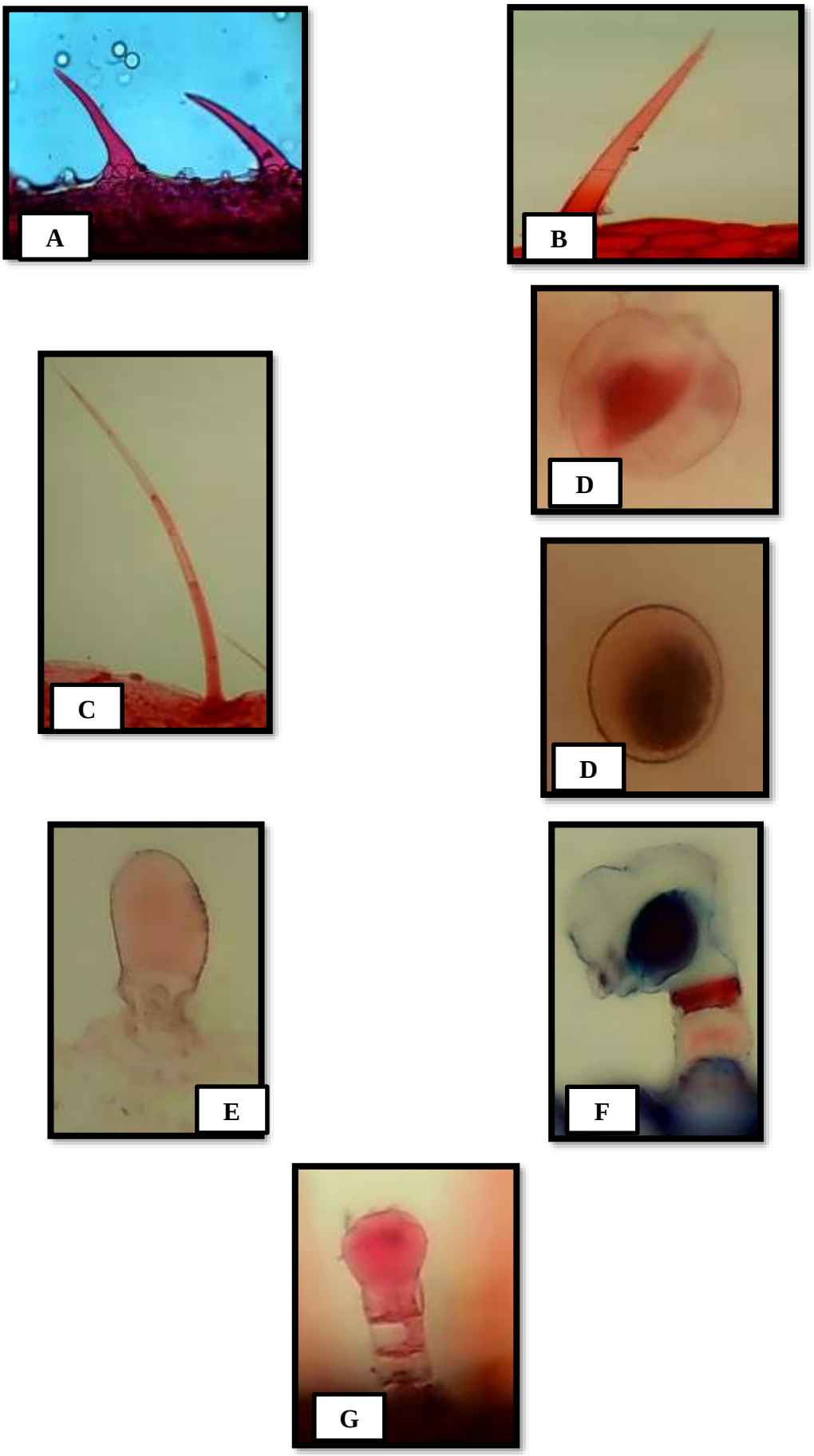


Figure 4. Trichomes of *Pelargonium graveolens* A, B, C: Non glandular hairs 100X, D-G: Glandular hairs 400X.

5. Conclusion

The result obtained by the study showed the importance of anatomical structure to *P. graveolens as a* medicinal plant. The study concluded that the surface epidermis of leaf and stem, and cross section of stem, petiole,petiole and leaf, besides glandular and non glandular hairs that have a relation to the production of volatile oil, especially glandular ones, druse crystals appear in different regions of the studied organs.

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